Latterich et al.

Serial No.:

09/458,779

Filed: Page 2

December 10, 1999

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-2 (cancelled)

- 3. (previously presented) An polynucleotide comprising a sequence encoding a vesicular fusion factor 2 protein (Vff2p), comprising SEQ ID NO:2, or conservative variations thereof, and further comprising a sequence encoding a heterologous target protein.
- 4. (previously presented) A polynucleotide comprising SEQ ID NO:1 or a sequence encodisng SEQ ID NO:2.
- 5. (previously presented) The polynucleotide of claim 3, wherein the <u>encoded</u> protein is about 32 kD.
- 6. (previously presented) The polynucleotide of claim 3, further comprising a promoter operatively linked to the sequence encoding the Vff2p.
- 7. (previously presented) The polynucleotide of claim 6 wherein the promoter is a promoter that functions in a host cell to direct transcription of the sequence encoding the Vff2p.

Claim 8 (cancelled)

Latterich et al.

Serial No.:

09/458,779

Filed:

December 10, 1999

Page 3

- 9. (previously presented) The polynucleotide of claim 8, further comprising a second promoter operably linked to the sequence encoding the target protein operably linked to a second promoter.
- 10. (original) The polynucleotide of claim 9, wherein the second promoter is a promoter that functions in the host cell to direct transcription of the target protein.
- 11. (original) The polynucleotide of claim 7, wherein the host cell is a yeast cell.
- 12. (previously presented) The polynucleotide of claim 11, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis.

## Claim 13 (cancelled)

- 14. (previously presented) A polynucleotide expression vector comprising a polynucleotide encoding a Vff2p comprising SEQ ID NO:2 or conservative variations thereof.
- 15. (previously presented) An expression vector comprising SEQ ID NO:1, or a sequence encoding SEQ ID NO:2.
- 16. (previously presented) The expression vector of claim 14, wherein the <u>encoded</u> protein is about 32 kD.

Inventors: Latterich et al.

Serial No.: 09/458,779

Filed: December 10, 1999

Page 4

17. (previously presented) The expression vector of claim 14, further comprising a promoter sequence operatively linked to the sequence encoding the Vff2p.

- 18. (previously presented) The expression vector of claim 17 wherein the promoter is a promoter that functions in a host cell to direct transcription of the sequence encoding the Vff2p.
- 19. (previously presented) The expression vector of claim 14, further comprising a sequence encoding a heterologous target protein.
- 20. (original) The expression vector of claim 19, wherein transcription of the target protein is directed by a second promoter.
- 21. (original) The expression vector of claim 20, wherein the second promoter is a promoter that functions in the host cell to direct transcription of the target protein.
- 22. (original) The expression vector of claim 18, wherein the host cell is a yeast cell.
- 23. (previously presented) The expression vector of claim 22, wherein the yeast is Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis.

Claim 24 (cancelled)

Latterich et al.

Serial No.:

variations thereof.

09/458,779

Filed: Page 5

December 10, 1999

25. (previously presented) A recombinant host cell comprising a yeast cell genetically altered to express a protein encoded by a polynucleotide sequence encoding a functional Vff2p, wherein the Vff2p comprises SEQ ID NO:2 or conservative

- 26. (previously presented) A host cell comprising SEQ ID NO:1, or a sequence encoding SEQ ID NO:2.
- 27. (previously presented) The host cell of claim 25, further comprising a sequence encoding a heterologous target protein.

Claim 28 (cancelled)

- 29. (previously presented) The host cell of claim 25, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis cell.
- 30. (previously presented) The host cell of claim 25, wherein the host cell lacks a functional protein involved in the secretory pathway and/or involved in the required cellular machinery for membrane fusion, other than Vff2p.

Inventors: Latterich et al.

Serial No.: 09/458,779

Filed: December 10, 1999

Page 6

31. (previously presented) A method for increasing cell growth of a yeast host cell, comprising introducing a polynucleotide sequence encoding Vff2p into the cell and culturing the cell, wherein the Vff2p comprises SEQ ID NO:2 or conservative variations thereof.

- 32. (previously presented) The method for increasing cell growth of a cell according to claim 31, wherein the host cell is cultured under conditions effective to allow expression of the Vff2p.
- 33. (previously presented) A method for increasing protein secretion from a yeast host cell, comprising introducing a polynucleotide sequence encoding Vff2p into the cell and culturing the cell, wherein the Vff2p comprises SEQ ID NO:2 or conservative variations thereof.
- 34. (previously presented) The method for increasing protein secretion from a cell according to claim 33, wherein the host cell is cultured under conditions effective to allow expression of the Vff2p.

## Claim 35 (cancelled)

36. (previously presented) An isolated vesicular fusion factor 2 protein comprising SEQ ID NO:2, or conservative variations thereof, and further comprising a heterologous target protein.

Inventors: Latterich et al.

Serial No.: 09/458,779

Filed: December 10, 1999

Page 7

37. (previously presented) A method of selecting for a yeast secretory mutant cell containing a polynucleotide sequence encoding a Vff2p operably linked to a promoter, wherein the Vff2p comprises SEQ ID NO:2, or conservative variations thereof, the method comprising growing the yeast secretory mutant cell at a restrictive temperature of about 32-37°C, wherein the restrictive temperature selectively favors mutant cell growth.

- 38. (original) The method of claim 37, wherein the temperature is at about 37°C.
- 39. (original) The method of claim 37, wherein the secretory mutant cell is sec17-1, sec18-1, bet1-1, sec22-2, uso1-1, pex3-1, sed5-1, cdc48-2, sec7-5, or ypt1-3.28.
- 40. (original) The method of claim 39, wherein the secretory mutant cell is sec17-1, sec18-1, bet1-1, sec22-2, uso1-1, or pex3-1.
- 41. (original) The method of claim 40, wherein the secretory mutant cell is sec18-1.
- 42. (previously presented) The method of claim 37, wherein the polynucleotide further comprises a sequence encoding a heterologous target protein operably linked to a second promoter.

Claims 43-45 (cancelled)

Latterich et al.

Serial No.:

09/458,779

Filed:

December 10, 1999

Page 8

46. (previously presented) The method of claim 31, 33 or 37, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis cell.

- 47. (currently amended) An isolated polynucleotide comprising a sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2, and further comprising a sequence encoding a heterologous target protein, wherein the vesicular fusion factor 2 protein increases Saccharomyces cerevisiae cell growth or protein [expression] secretion.
- 48. (currently amended) An isolated polynucleotide comprising SEQ ID NO:1 encoding a vesicular fusion factor 2 protein that increases Saccharomyces cereviae cell growth or protein [expression] secretion, and further comprising a sequence encoding a heterologous target protein.
- 49. (currently amended) A polynucleotide expression vector comprising a polynucleotide encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2, wherein the vesicular fusion factor 2 protein increases *Saccharomyces cerevisiae* cell growth or protein [expression] secretion.
- 50. (previously presented) A recombinant host cell comprising a *Saccharomyces cerevisiae* cell genetically altered to express a protein encoded by a polynucleotide sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2.

Latterich et al.

Serial No.:

09/458,779

Filed: Page 9

December 10, 1999

51. (previously presented) A method for increasing cell growth of a Saccharomyces cerevisiae host cell, comprising introducing a polynucleotide sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2 into the cell and culturing the cell.

- 52. (previously presented) A method for increasing protein secretion from yeast host cell, comprising introducing a polynucleotide sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2 into the cell and culturing the cell.
- 53. (previously presented) A composition comprising two vectors, the first vector comprising a polynucleotide encoding the vesicular fusion factor 2 protein and the second vector comprising a polynucleotide encoding a heterologous target protein.
- 54. (previously presented) The composition of claim 53, wherein the polynucleotide encoding the heterologous target protein further comprises a signal sequence.